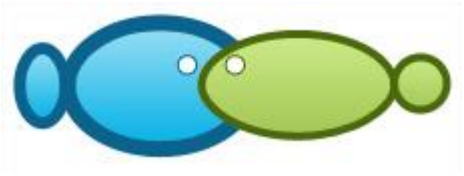

Dynamics of age changes for the biochemical parameters of sperm production in Amur carp (*Cyprinus rubrofuscus*) of different geneses

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Abstract. The aim of this work was to study the physiological characteristics of Amur carp (*Cyprinus rubrofuscus*) ejaculate, the activity of mitochondrial respiratory chain enzymes CcO and SDH, as well as sperm survival and evaluation of dynamic parameters of sperm of age 8+ and 9+ Amur carp of different genetic origins (produced from cryopreserved and native sperm). The study showed that the total activity of sperm of local Amur carp males decreased by 6.9%, while in cryo-males it increased by 2.4%. At the same time, the number of sperm with straight line velocity in age 8+ cryo-males was 3.1% higher than in local ones. This value in age 9+ cryo-males was 5.6% higher than in local males ($p<0.05$). The number of non-motile spermatozoa in cryo-males decreased by 3.4%, while in local males it increased by 6.9%. Survival of diluted sperm of males produced by cryotechnology increased by 67.4%, while that of local males by 72.7%, and undiluted sperm by 44.4 and 23.3%, respectively. However, there was a decrease in SDG and CcO by 16.4 and 65.2% in cryo-males, and by 31.0 and 71.54% in males from the local stock, respectively. The analyses of soluble sperm proteins of age 8+ and 9+ carp showed zones of globulins typical for electrophoretic separation of proteins: γ-, β- and α-, albumin and prealbumins.
Key Words: albumin, cytochrome oxidase, globulins, sperm activity, succinate dehydrogenase.

Introduction. The development of aquaculture requires the breeding and preservation of the gene pool of a variety of cultured species. Therefore, there is a need to study the biological characteristics as well as issues of the conservation and rational use of the gene pool of the Amur carp (*Cyprinus rubrofuscus*) population available in the aquaculture of Ukraine. Thus, there is a problem of the formation of pure lines of Amur carp in aquaculture for the possibility of their further use in industrial hybridization for commercial cultivation and selective breeding (Kolisnyk et al 2014a). Shipping of Amur carp brood stock from its native range (China) to Ukraine is very expensive and, therefore, unprofitable. Consequently, there is a need for non-traditional, new, and modern methods in fish farming practice, including the use of cryopreserved sperm to reproduce the population of Amur carp (Kolisnyk et al 2015). In 2011, "blood refreshment" of Amur carp brood stock cultured for a long time in pond conditions was performed by using defrosted sperm from Amur carp males from the native water body, the Amur River basin. For this purpose, a number of selection works were carried out on the reproduction of carp by natural spawning of existing breeding material and the use of cryopreserved sperm (Kopeika et al 2011; Kolisnyk et al 2014b).

Cryopreservation of fish sperm for the purpose of preservation and further use of the genetic material of the most valuable brood fish is increasingly being used in aquaculture (Kononenko 2016; Betsy & Kumar 2020). Currently, cryopreservation is used to preserve the genetic diversity of rare and endangered fish species, carry out selective breeding works, and supply hatcheries with sperm in order to produce various crosses and hybrids (Horvath et al 2012; Liu et al 2016). The use of cryopreserved fish sperm in modern aquaculture necessitates a more detailed study of its effect and long-term (e.g.,

tens of years) storage at ultra-low temperatures (Bezusiy et al 2011). Since works were previously carried out on the reproduction of pure lines of Amur carp from cryopreserved sperm and native sperm, the question remains of a more detailed study on the use of these pure lines as brood fish in industrial hybridization. The selection of brood fish with high quality and fertilizing ability of sperm is of great importance in selective breeding (Ostapiv 2008a). The qualitative characteristics of spermatozoa are one of the most important factors under which the process of fertilization takes place with the subsequent development of the embryo (Yaremchuk et al 2015). Researchers found not only interspecies, individual, and seasonal differences in sperm quality, but also daily fluctuations within the same animal (Yablonsky et al 2006). All this should be taken into account, since certain external factors have a direct effect on the body, i.e., they can accelerate, slow down or completely stop the development of gametes (spermatogenesis).

Motility is one of the most important characteristics associated with the fertilizing ability of spermatozoa, indicating their viability and structural integrity. Therefore, the study of motility is an integral part of sperm analysis. The assessment of sperm motility has become widely used in reproductive technology, as this method allows determining the quality of the resulting gametes, detect abnormalities, and prevent inefficiencies in fertilization. A detailed analysis of the motility and evaluation of the dynamics of the movement of sperm from brood fish allows forming sperm according to categories of motility, distinguishing samples with fast and rectilinear movement, slow rectilinear movement, non-linear movement and samples with completely immobile sperm (Fauvel et al 2010; Yaremchuk & Sharan 2012; Yurchak et al 2018).

Among different studies, an important direction is the study of the qualitative characteristics of ejaculates – microscopic assessment of sperm density and sperm activity, determination of sperm concentration, sperm survival after ejaculates thaw. However, these methods are indicative and do not show the fertilizing ability of sperm. Enzymes can be used to assess sperm quality, as the most sensitive and genetically determined tests (Ostapiv 2008b; Shostia 2014). Sperm membranes are susceptible to peroxidative damage by excess reactive oxygen species (ROS). The effectiveness of the functioning of the enzymatic antioxidant defense system, the key enzymes of which are superoxide dismutase, catalase (CAT), and glutathione peroxidase, is important for preserving structural integrity, sperm survival, and preventing lipid peroxidation (LPO) processes in it. It has been established that the quality of sperm is interrelated with the state of antioxidant protection of the body, and the disorder of the mechanisms of regulation of the processes of free radical oxidation of lipids can be one of the pathogenetic factors of impaired reproductive function of brood animals (Tsekhmistrenko & Koberska 2013). In particular, one of the antioxidant enzymes is catalase (CAT), which provides neutralization of hydrogen peroxide by splitting H_2O_2 with the formation of water and oxygen (Lukyanova et al 1982). Some studies showed the positive effect of catalase activity on mobility, viability and lipid peroxidation (Foote 1962; Kankofer et al 2005; Pagl et al 2006; Bansal & Cheema 2016; Singh et al 2020).

A direct relationship between the activity of oxidative enzymes and the fertilizing ability of sperm has been established, and it is more pronounced with the activity of succinate dehydrogenase (SDH). A direct relationship between the activity of many dehydrogenase and cytochrome oxidase (CcO) enzymes of sperm with indicators of its quality and fertilizing ability was shown, which served as the basis for using the activity of these enzymes as an indicator of sperm quality (Ruiz-Pesini et al 1998; Barbagallo et al 2020; Linkevich et al 2014). Scientists have shown a connection between SDH activity of bull sperm and fertilizing ability. In particular, a decrease in the fertilizing ability of bull sperm was observed with a decrease in enzyme activity in the ejaculate (Söderquist et al 1991; Dudchak & Sharan 2010).

Thus, one of the most important factors responsible for successful reproduction is the quality of gametes. Therefore, the aim of this study was to investigate the physiological characteristics of the ejaculates' respiratory and restorative capacity, the activity of the enzymes of the respiratory chain of the mitochondria CcO and SDH, as well

as the survival of sperm and the evaluation of the dynamic parameters of the sperm of age 8+ and 9+ Amur carp of different genetic origin.

Material and Method. The object of the study were two groups of Amur carp males reared in ponds of the State Enterprise "Lviv Research Station" of the Institute of Fisheries of the National Academy of Agrarian Sciences (Lviv region). The control had local carp, age 8+ and 9+ males produced from native sperm of local stocks of Amur carp, which are descendants of the brood stock brought to Ukraine from the Russian Far East (Lake Khanka, Amur River basin) in the 1976 and have passed eight generations of reproduction of age 4+ fish. The experimental group was represented by cryo-males, age 8+ and 9+, produced from the defrosted sperm of Amur carp brought to Ukraine from Lake Khanka, which was frozen in 1976 (Bezusiy et al 2011; Kolisnyk et al 2014a,b). Sperm of Amur carp males for the study was collected during spawning campaigns in May 2019 and 2020. In total, sperm was collected from 16 males including four age 8+ males of the experimental group in 2019 and four age 9+ males of the experimental group in 2020, as well as four age 8+ males of the control group in 2019 and four age 9+ males of the control group in 2020. All males used for the study were clinically healthy and corresponded to the "elite" class based on their growth, development, and external features. Sperm was collected during the spawning campaign by gently squeezing the abdomen of the fish. Approximately 1 mL of sperm samples from each male were placed into 2 mL Eppendorfs. The cooled sperm was transported in a special thermos to the laboratory of the Institute of Animal Biology (Lviv). Spermatozoa were activated with a phosphate-buffered saline (NaCl – 0.8 g, KCl – 0.02 g, Na_2HPO_4 – 0.11 g, KH_2PO_4 – 0.02 g, $MgCl_2$ – 0.01 g, H_2O up to 100 mL).

The following sperm parameters were determined under a microscope: total motility, percentage of sperm with progressive motility and circular movement, quantity of non-motile spermatozoa. A basic semen analysis assessed the quantity and quality of each sample by assessing semen volume, pH, macro movement, sperm concentration, sperm motility (including percentages total motility, progressive motility, rapid-, medium- and slow-swimming spermatozoa) and sperm vitality. Sperm kinematics were assessed using a CASA (Computer Assisted Semen Analysis) system, consisting of a phase-contrast Olympus CX 31 microscope (Olympus, Japan) connected to the SpermVision™ analyzer (Minitüb GmbH, Tiefenbach, Germany): straight line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), linearity (LIN=VSL/VCL), straightness (STR=VSL/VAP), and wobble (WOB = VAP/VC) (Ehlers et al 2011). Also, the general analysis of the following sperm parameters was carried out: general activity, percent of spermatozoa with straight line velocity and curvilinear velocity, number of non-motile spermatozoa. The following were also determined: spermatozoa survival time (hours) at a temperature of 22-24°C until cessation of progressive motility, activity of succinate dehydrogenase (SDH; U/h × 0.1 mL sperm) and cytochrome c oxidase (CcO, U/h × 0.1 mL sperm), oxygen consumption by a polarographic assay (ng-atom O/min × 0.1 mL sperm) at a temperature of 38.5°C in a phosphate-buffered saline (NaCl – 0.8 g, KCl – 0.02 g, Na_2HPO_4 – 0.11 g, KH_2PO_4 – 0.02 g, $MgCl_2$ – 0.01 g, H_2O up to 100 mL) (Chukhriy & Klevets 1978).

The method of determining the SDH activity is based on the ability of 2-, 3-, 5-, triphenyltetrazolium chloride to be reduced to red formazol under the action of the enzyme. The studied mixture acquires a pink color, the intensity of which depends on SDH activity. The α naphthol and paraphenylenediamine form a blue or bluish-violet product in the presence of CcO – indophenol blue, the color intensity of which is proportional to the activity of the enzyme.

The content of soluble protein fractions was determined by vertical electrophoresis in plates of 7.5% polyacrylamide gel (PAAG) (Gaal et al 1982). Samples for electrophoresis were prepared as follows: 0.1 mL of sperm was diluted with 1:12 electrode buffer (pH 8.5); 0.1 mL of the sample was mixed with a similar quantity of 40% sucrose; 0.02 mL (~150-200 µg protein) of the obtained sample was placed in wells of the concentrating gel. A marker dye (0.01% bromophenol blue solution) was added to the electrode buffer before diluting the samples. After electrophoresis, the gels were

fixed and simultaneously stained in 12.5% trichloroacetic acid, with 0.25% aqueous solution of Coumassi R-250.

Due to the fact that age 9+ males are usually culled out in aquaculture and, taking into account significant differences between the spermatozoa with progressive motion and the number of non-motile spermatozoa in samples of age 9+ males, using microscopic examination by computerized CASA system and the Sperm Vision program, the quality of sperm was assessed more objectively and optimally over time (Figure 1).

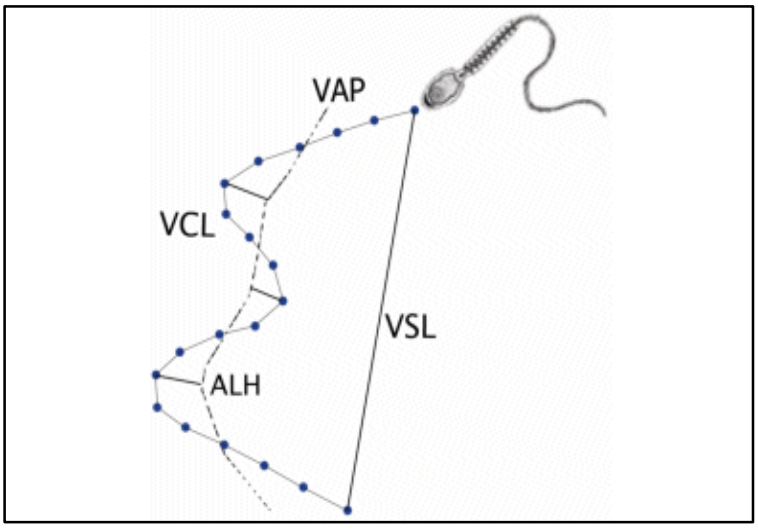

Figure 1. Kinematic parameters of sperm of age 9+ Amur carp (*Cyprinus rubrofuscus*) males; VSL – straight line velocity; VCL – curvilinear velocity; VAP – average path velocity.

CAT isozymes were detected after electrophoresis in 7.5% PAAG. Gel plates were stained according to Woodbury et al (1971): soaked for 45 min in distilled water, saturated with 0.003% hydrogen peroxide solution for 10 min, washed three times with water and incubated at room temperature in the dark for 15 min in a medium containing 1% solution of potassium ferricyanide (III) and iron chloride. After staining, the sites of localization of CAT proteins appear with bright yellow stripes on a blue-green background. Analysis of soluble protein fractions and CAT isozyme content (%) was performed using the TotalLab TL120 software (Nonlinear Inc, Durham NC, USA).
Statistical analysis of the obtained data was performed in MS Excel. All determined parameters were compared between two groups (cryo- and native) of carps using a *t*-test ($p<0.05$, $p<0.01$, $p<0.001$).

Results. The total number of active spermatozoa in age 8+ males varied from 90.7 in cryo-males to 94.4% in local males. At the same time, the number of sperm with progressive motility was 3.1% higher in cryo-males compared to local males.

The number of active spermatozoa in age 9+ cryo-males was 5.6% higher than in local males, and this difference was statistically significant ($p<0.05$). At the same time, the largest number of spermatozoa with progressive motility was recorded in cryo-males (70.5±2.3), which was 12.2% higher than in local males. Cryo-males of this age also had twice as many non-motile spermatozoa (Table 1).

Table 1

Sperm physiological parameters of Amur carp (*Cyprinus rubrofuscus*) from different origins

Group of Amur carp (n=4)	Parameters (mean±SE)			
	AS (%)	PM (%)	CM (%)	NM (%)
	Age 8+			
Cryo-males	90.7±3.8	72.5±2.9	18.2±0.9	9.3±1.8
Local males	94.4±2.1	69.4±2.5	25.0±0.4***	5.6±0.8
	Age 9+			
Cryo-males	93.1±4.8	70.5±2.3*	22.6±0.7	6.9±0.9
Local males	87.5±3.9	58.3±2.5	29.2±0.4***	12.5±1.2

Note: AS – active spermatozoa; PM – spermatozoa with progressive motility; CM – spermatozoa with circular movement; NM – non-motile spermatozoa; * - differences significant at $p<0.05$; ** - differences significant at $p<0.01$; *** - differences significant at $p<0.001$.

Comparing the quality of sperm in age 8+ and 9+ carp, it should be noted that the total motility of spermatozoa of cryo-males increased by 2.4%, while that of local males decreased by 6.9%. At the same time, the share of spermatozoa with progressive motion dropped by 2 and 11.1%, respectively, in cryo- and local males. The number of sperm with circular movement increased by 4.4 and 4.2%, respectively. The number of non-motile spermatozoa was the most variable among the studied parameters, decreasing by 3.4% in cryo-males and increased by 6.9% in local males. As a result, the number of non-motile spermatozoa in age 9+ cryo-males was almost twice as low compared to age 8+ cryo-males.

The straight line velocity of spermatozoa (VSL) in age 9+ males showed that the average value of this dynamic characteristic of sperm in the studied groups was 24.39 and 29.38 μm sec^{-1}, respectively, in cryo-males and local males (Table 2).

Table 2

Kinematic parameters of sperm obtained using the CASA system (mean±SE)

Parameters	*Sperm samples of age 9+ males (n=4)*	
	cryo-males	*local males*
VSL – straight line velocity (μm sec^{-1})	24.3±1.8	29.3±2.1
VCL – curvilinear velocity (μm sec^{-1})	57.6±3.6	71.1±4.3
VAP – average path velocity (μm sec^{-1})	29.5±2.3	40.7±3.2
STR – straightness, (VSL/VAP, %)	74.1±4.2	72.5±3.9
LIN – linearity, (VSL/VCL, %)	51.3±2.6	41.2±2.3
WOB – wobble (VAP/VCL, %)	69.0±4.3	57.0±3.5

The VCL of spermatozoa was significantly higher by 13.43% in local males. A higher result in the same study group was obtained when studying STR. This value indicates the best fertilizing ability of sperm. The VAP in cryo-males was 29.52 and 40.71 μm sec^{-1} in local males. The WOB and the frequency of oscillating movements of sperm did not show a significant difference between the two groups.

The survival time of diluted sperm of age 8+ cryo-males at a temperature of 22-24°C was 23.5±3.9 h and was higher by 23.5% compared to that of local carp males (Figure 2).

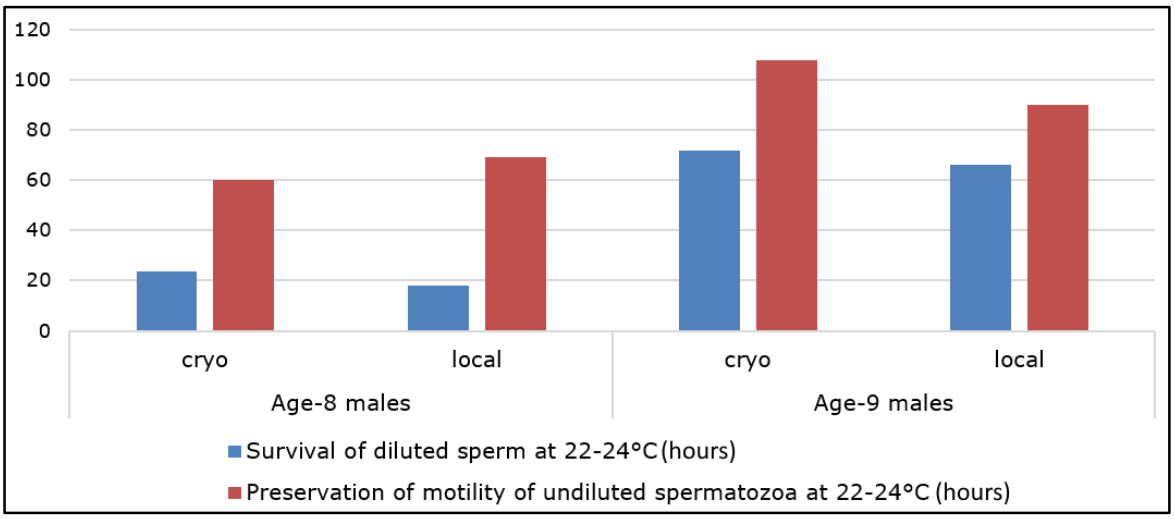

Figure 2. Survival time of spermatozoa in different physical conditions (n=4).

However, the preservation of motility of undiluted sperm at a temperature of 22-24°C (ability to restore the motility of undiluted sperm) was shorter by 9 h (13.1%) in sperm of local males, compared with analogues obtained from cryopreserved sperm. Survival of both diluted and undiluted sperm at 22-24°C in age 9+ cryo-males was higher by 9% and 20%, respectively, compared to that in males of local carp. At the same time, the survival time of diluted sperm in age 9+ males compared to age 8+ males increased by 67.4% in cryo-males and 72.7% in local males, while that of undiluted sperm increased by 44.4% and 23.3%, respectively. However, these differences were not statistically significant.

The activity of oxidative enzymes of sperm showed that SDH activity in age 8+ local males was higher by 6.4% compared to cryo-males. CcO activity in the sperm of both groups of males did not differ significantly and was in the range of 52.7-53.7 U/h×0.1 mL of sperm. At the same time, age 9+ males had a decrease in SDH and CcO activities compared to age 8+ fish by 16.4% and 65.2% in cryo-males, and by 31.0% and 71.5% in local males, respectively (Figure 3).

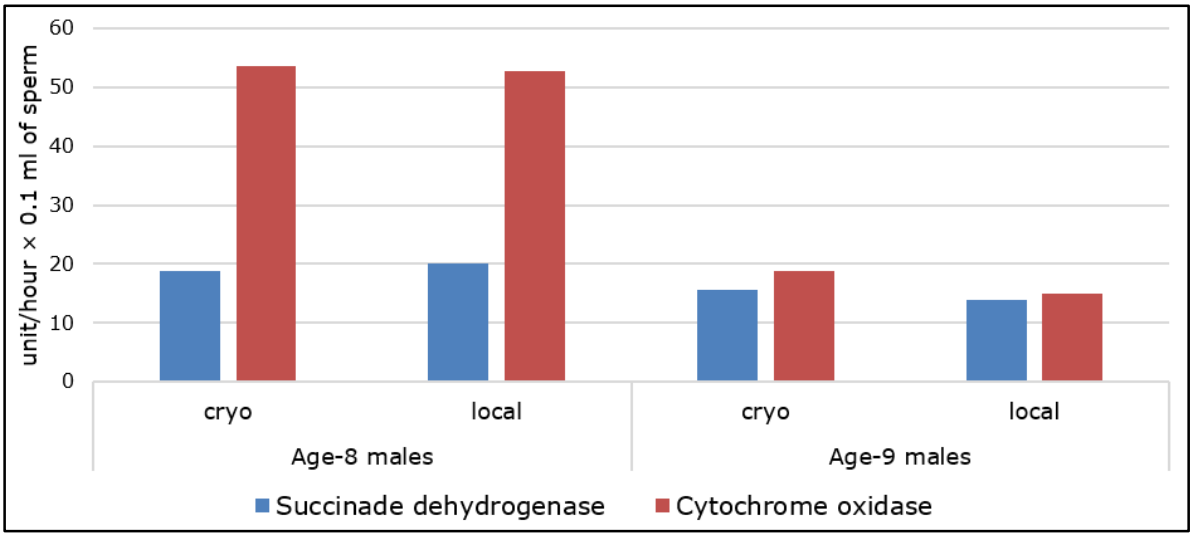

Figure 3. Activity of oxidative enzymes in milt of Amur carp (*Cyprinus rubrofuscus*) males of different geneses (n=4).

The studies of soluble sperm proteins of age 8+ and 9+ males showed zones of globulins typical for electrophoretic separation of proteins (relative to blood serum): γ-, β-, and α-, albumin and prealbumins (Figure 4).

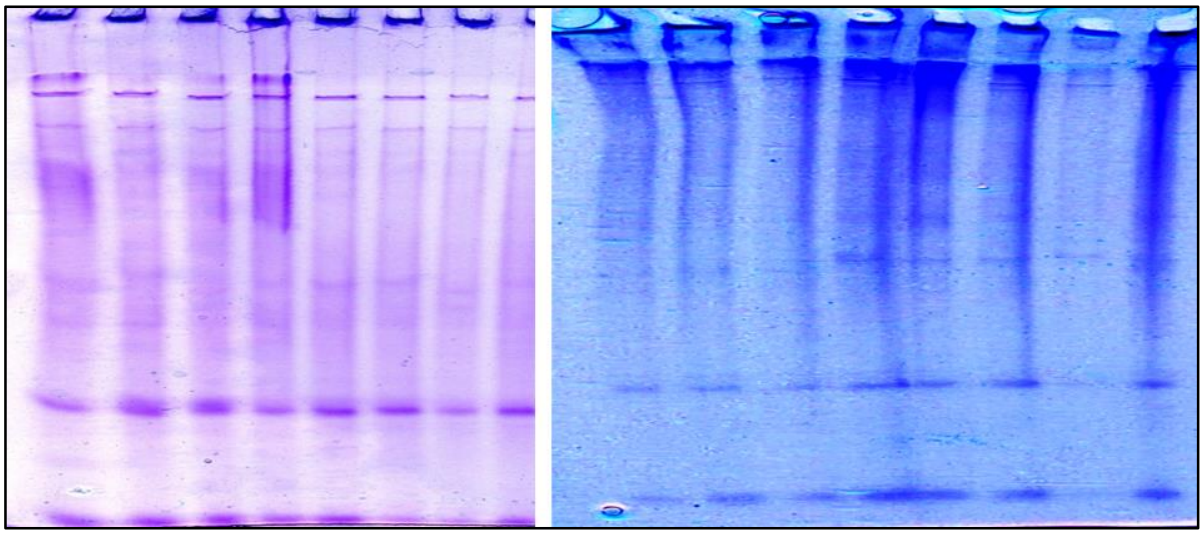

Figure 4. Electropherograms of fractions of soluble proteins of age 8+ (left) and 9+ (right) Amur carp (*Cyprinus rubrofuscus*) sperm.

The content of γ- and β-globulin in the sperm of age 8+ carp was 7.9% ($p<0.05$) and 2.1% higher, respectively, in cryo-males compared to local males. The content of α-globulins in the sperm did not differ significantly in different groups and was in the range of α_3 – 5.5-6.2%, α_2 – 2.4-3.0 % and α_1 — 3.8-4.3%. A similar result was also obtained for the albumin content: the value was in the range of 8.8-10.5%. A larger difference was found in the content of prealbumins: their maximum content (24.9±1.1%) was found in the sperm of local males, 6.2% ($p<0.05$) higher than in cryo-males (18.7±1.5%) (Table 3).

The protein content of the γ-globulin and albumin zone in age 9+ carp was significantly higher by 6.8% ($p<0.05$) and 3.7% ($p<0.001$), respectively, in the sperm of cryo-males compared to local carp. The content of β-, α_3- and α_2-globulins did not differ significantly between groups and was in the range of 4.6-6.3%, 2.7-3.2%, and 4.1-4.2%, respectively. A significantly greater difference was found for proteins of α_1-globulin and prealbumin zone: their higher content (3.7±0.76 and 28.6±1.44%, respectively) was observed in the sperm of local males ($p<0.05$) compared with cryo-males.

Table 3

The content of fractions of soluble proteins of Amur carp (*Cyprinus rubrofuscus*) sperm of different generations (mean±SE; n=4)

Protein fractions (%)	*Age 8+ males*		*Age 9+ males*	
	cryo	*local*	*cryo*	*local*
γ-globulins	49.0±1.44*	41.1±2.87	55.7±1.81*	48.9±2.20
β-globulins	8.6±0.88	6.5±0.86	4.6±0.91	6.3±0.62
α_3-globulins	6.2±0.47	5.5±0.38	3.2±0.42	2.7±0.79
α_2-globulins	2.4±0.28	3.0±0.53	4.2±0.45	4.1±0.97
α_1-globulins	3.8±0.32	4.3±0.73	1.4±0.49	3.7±0.76
Albumin	8.8±0.64	10.5±0.88	8.3±0.35***	5.7±0.74
Prealbumin	18.7±1.50	24.9±1.10*	22.7±1.63	28.6±1.44*

Note: * - significant differences at $p<0.05$; ** - significant differences at $p<0.01$; *** - significant differences at $p<0.001$.

The study of CAT isozymes showed that age 8+ Amur carp sperm displayed one catalytically active catalase band. Regardless of sperm origin, their content was the same, 25% (Table 4).

Table 4

The content of catalase (CAT) isozymes in sperm of Amur carp (*Cyprinus rubrofuscus*) males, (mean±SE, n=4)

Sperm samples	*Age 8+ males*	*Age 9+ males*	
	Content of CAT isozymes in sperm (%)		
	CAT	*CAT2*	*CAT1*
Cryo-males	25.0±9.10	86.6±7.61	13.4±7.61
Local males	25.0±6.50	41.3±3.48	58.7±3.48

The study of CAT isozymes in the ejaculates of age 9+ Amur carp showed two bands of active proteins of the CAT_1 and CAT_2 enzymes, but their contents were different and depended on growing conditions and genetic origin of males. The content of CAT_2 isoforms was significantly higher by 45.3% ($p<0.001$) and CAT_1 was correspondingly lower in sperm of cryo-males compared to local males.

Discussion. In recent years, scientists have been conducting an intensive search for auxiliary biological tests that would allow speeding up and increasing the accuracy of selective breeding techniques and methods for assessing the exterior, reproductive and breeding qualities of animals. The full implementation of measures to preserve breeding resources of livestock is ensured by the creation of gene pool herds in combination with cryopreservation and long-term storage of genetic material in cryobanks. The main purpose of the bank is the accumulation and long-term storage of genetic resources of all types of livestock, as well as the implementation of a complex of organizational and technological measures for the preservation and rational use of the existing gene pools in Ukraine (Gladiy et al 2018).

At the same time, it is important to periodically monitor the quality of sperm to establish the feasibility of its further storage and the possibility of using it in the selective breeding process. This is possible due to the determination of the physiological and biochemical characteristics of sperm, as well as factors affecting the process of spermatogenesis, which will allow determining the functional properties of sperm and subsequently improving the quality of sperm production. The intensity of biochemical processes was found to largely determine the reproductive capacity of males and the fecundity of females, which in turn affects the quality of their offspring. The biochemical composition of sperm depends on individual characteristics and the level of nutrition. At the same time, the quality of fish ejaculates is affected by numerous environmental factors (feeding, keeping conditions, thermal regime, season) in addition to genetic factors (breed, line) (Linkevich et al 2014).

Animal semen contains high concentrations of protein. Interacting with other substances, sperm proteins form such complexes as lipoproteins, phosphoproteins, nucleoproteins, glycoproteins, etc. Sperm proteins contain high levels of arginine, leucine, lysine, cystine, and glutamic acid, and among lipids, high lecithin, which is characterized by a high phosphorus content (Yablonsky et al 2006). In the body of animals, including fish, proteins are in a dynamic state, which is ensured by the processes of continuous synthesis and decay. The latter are determined genetically and depend on living conditions. Unlike mammals, the share of amino acids in fish only in the substrate supply of energy metabolism can reach 50-90%. This is explained by the action of many extreme environmental factors and requires the rapid extraction of metabolic energy from readily available substrates to ensure the normal functioning of their body. The analysis of literary sources indicates a certain relationship between the parameters of sperm production and the fertilizing ability of sperm (Siratsky 1994).

The analysis of the age dynamics according to physiological parameters showed that sperm survival, activity of sperm oxidative enzymes, and the content of catalase

isozymes in sperm were higher in age 9+ males. Therefore, their fertilization capacity was higher. No significant differences were found in the content of soluble protein fractions of carp sperm. It is obvious that one of the factors for the difference is the pre-spawning feeding, which took place in slightly different periods compared to age 8+ fish, as well as the influence of environmental factors during fattening.

Therefore, the presented analysis of the quality of sperm production testifies to the ability of age 9+ males evaluated based on hereditary features to produce high quality sperm with high activity of oxidative enzymes. In terms of reproductive performance, age 9+ males were not inferior to age 8+ males and, accordingly, they were used in the spawning campaign. The reasons for individual differences in the quality of ejaculates can be the live weight, conditions of keeping, the size of gonads, and the hormonal state of the brood fish.

Based on the results of the study on the quality of ejaculates of age 8+ and 9+ carp of various origins, it is possible to assert the positive impact of the use of cryotechnologies in aquaculture.

The progress in the field of cryobiology, developmental biology, population genetics and fish breeding, as well as in other areas of science, allows creating new aquaculture technologies that are characterized by higher economic efficiency and stability. The most important direction of this activity is the formation of gene pool collections – both living and those stored in the form of cryopreserved reproductive material, mainly sperm. Currently, cryotechnologies are strategically important, including anti-crisis technologies, for solving problems related to the preservation of genetic biodiversity of fish.

Conclusions. The comprehensive study and analysis of the sperm production of Amur carp males showed that the quality of sperm of age 8+ and 9+ males corresponded to their physiological state and was within the normative values. In general, the obtained data indicate high values of reproductive parameters and high fertilization capacity of both age males, and the possibility of their use in selective breeding. According to the general activity of sperm, age 8+ cryo-males prevailed, with 96.2%. A higher straight line velocity of sperm was also recorded in age 8+ males that were grown from cryopreserved sperm. These results indicate the better fertilizing capacity of this sperm. The survival rate of both diluted and undiluted sperm of age 9+ carp grown from cryopreserved sperm showed a tendency to increase, compared to males produced from native sperm. In age 8+ fish, increased values of preservation of motility of undiluted sperm (ability to restore motility of undiluted sperm) and SDH activity were manifested in carp grown from native sperm. In age 9+ fish, an increased activity of SDH and CcO in sperm was manifested in carp grown from cryopreserved ejaculates. The obtained results indicate the possible reasons for the variability of the physiological parameters of the sperm and, accordingly, their fertilizing ability, which can include individual characteristics, age and season, conditions of keeping and feeding, because the level of physiological processes in the body is closely related to conditions of the external environment.

Conflict of Interest. The authors declare that there is no conflict of interest.

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